#2   OIPE

RAW SEQUENCE LISTING   DATE: 07/24/2001
PATENT APPLICATION: US/09/901,419   TIME: 10:53:13

Input Set : **A:\Umo15311.app**
Output Set: **N:\CRF3\07242001\I901419.raw**   **ENTERED**

```
 3 <110> APPLICANT: The Curators of the University of Missouri
 5 <120> TITLE OF INVENTION: LARGE SCALE EXPRESSION AND PURIFICATION OF RECOMBINANT
 6       PROTEINS
 8 <130> FILE REFERENCE: UMO1531.1
C--> 10 <140> CURRENT APPLICATION NUMBER: US/09/901,419
C--> 11 <141> CURRENT FILING DATE: 2001-07-09
13 <150> PRIOR APPLICATION NUMBER: 60/218,125
14 <151> PRIOR FILING DATE: 2000-01-13
16 <160> NUMBER OF SEQ ID NOS: 2
18 <170> SOFTWARE: PatentIn Ver. 2.1
20 <210> SEQ ID NO: 1
21 <211> LENGTH: 4087
22 <212> TYPE: DNA
23 <213> ORGANISM: Bos taurus
25 <220> FEATURE:
26 <221> NAME/KEY: CDS
27 <222> LOCATION: (268)..(3180)
29 <220> FEATURE:
30 <221> NAME/KEY: sig_peptide
31 <222> LOCATION: (268)..(363)
33 <220> FEATURE:
34 <221> NAME/KEY: misc_feature
35 <222> LOCATION: (3178)
36 <223> OTHER INFORMATION: A Poly (H) affinity tag comprising 6 His residues
37       have been inserted at the C-Terminus end of the
38       coding region of the protein
40 <400> SEQUENCE: 1
41 gaattcggga gaagccatca ccccgggtct tttttcacat ccagcccatg cagaccgatc 60
43 ggccagctca accagagctg ccactgatct tccacactta agcaaaccac accagtgagt 120
45 ggcgaacatc aactcgtgct tgaaaaatac caacttggag cccggtttga gaagctacat 180
47 cagagtctcg agatgcgacg ctacaatctg cagttttcac tagcttccca gtaggttggg 240
49 acagttggaa ctctgccatt gcccagc atg ctg cag ttc agt ctg tca ccc acc 294
50                                 Met Leu Gln Phe Ser Leu Ser Pro Thr
51                                  1               5
53 ttg tcg atg gga ttt cac gtg ata gcc atg gtg gct ctc ttg ttt tcc   342
54 Leu Ser Met Gly Phe His Val Ile Ala Met Val Ala Leu Leu Phe Ser
55  10              15                  20                  25
57 cat gtg gac cat ata agt gct gag aca gaa atg gaa gga gaa ggc aac   390
58 His Val Asp His Ile Ser Ala Glu Thr Glu Met Glu Gly Glu Gly Asn
59              30                  35                  40
61 gag act ggc gag tgt act ggc tcc tat tac tgt aag aag ggg gtg att   438
62 Glu Thr Gly Glu Cys Thr Gly Ser Tyr Tyr Cys Lys Lys Gly Val Ile
63              45                  50                  55
65 tta ccc att tgg gag ccc cag gac cct tcc ttt gga gac aaa att gct   486
66 Leu Pro Ile Trp Glu Pro Gln Asp Pro Ser Phe Gly Asp Lys Ile Ala
67          60                  65                  70
69 aga gcg act gtg tat ttt gtg gcc atg gtc tac atg ttt ctt gga gtc   534
```

RAW SEQUENCE LISTING   DATE: 07/24/2001
PATENT APPLICATION: US/09/901,419 TIME: 10:53:13

Input Set : A:\Umol5311.app
Output Set: N:\CRF3\07242001\I901419.raw

```
70 Arg Ala Thr Val Tyr Phe Val Ala Met Val Tyr Met Phe Leu Gly Val
71     75                  80              85
73 tca atc att gct gac cgg ttc atg tcc tct ata gaa gtc atc acg tct    582
74 Ser Ile Ile Ala Asp Arg Phe Met Ser Ser Ile Glu Val Ile Thr Ser
75 90                  95              100                 105
77 caa gag aaa gaa atc acc ata aag aaa ccc aat gga gag acc acc aag    630
78 Gln Glu Lys Glu Ile Thr Ile Lys Lys Pro Asn Gly Glu Thr Thr Lys
79                 110                 115                 120
81 aca act gtg agg atc tgg aat gag aca gtg tcc aac ctg acc ttg atg    678
82 Thr Thr Val Arg Ile Trp Asn Glu Thr Val Ser Asn Leu Thr Leu Met
83             125                 130                 135
85 gcc ctg ggg tct tca gct cca gag att ctc ctt tca gta atc gag gtg    726
86 Ala Leu Gly Ser Ser Ala Pro Glu Ile Leu Leu Ser Val Ile Glu Val
87             140                 145                 150
89 tgt ggc cat aac ttc act gca gga gac ctt ggc cct agc acc atc gtg    774
90 Cys Gly His Asn Phe Thr Ala Gly Asp Leu Gly Pro Ser Thr Ile Val
91     155                 160                 165
93 ggg agt gct gca ttc aac atg ttc atc atc att gcc ctt tgt gtg tat    822
94 Gly Ser Ala Ala Phe Asn Met Phe Ile Ile Ile Ala Leu Cys Val Tyr
95 170                 175                 180                 185
97 gtc gtc ccg gat ggg gag aca agg aag atc aag cat ctg cgt gtg ttc    870
98 Val Val Pro Asp Gly Glu Thr Arg Lys Ile Lys His Leu Arg Val Phe
99                 190                 195                 200
101 ttt gtg aca gca gca tgg agc atc ttt gcc tat acc tgg ctt tac atc   918
102 Phe Val Thr Ala Ala Trp Ser Ile Phe Ala Tyr Thr Trp Leu Tyr Ile
103             205                 210                 215
105 att ttg tct gtc agc tcc cct ggg gtc gtg gag gtc tgg gaa ggt ttg   966
106 Ile Leu Ser Val Ser Ser Pro Gly Val Val Glu Val Trp Glu Gly Leu
107             220                 225                 230
109 ctt act ttc ttc ttc ttc ccc atc tgc gtt gtg ttt gct tgg gtg gca   1014
110 Leu Thr Phe Phe Phe Phe Pro Ile Cys Val Val Phe Ala Trp Val Ala
111     235                 240                 245
113 gac agg agg ctt ctg ttt tac aag tat gtc tac aag agg tat cgg gct   1062
114 Asp Arg Arg Leu Leu Phe Tyr Lys Tyr Val Tyr Lys Arg Tyr Arg Ala
115 250                 255                 260                 265
117 ggc aag cag agg gga atg att att gaa cac gaa gga gac agg cca tct   1110
118 Gly Lys Gln Arg Gly Met Ile Ile Glu His Glu Gly Asp Arg Pro Ser
119                 270                 275                 280
121 tcc aag aca gaa att gaa atg gat ggg aaa gtg gtc aat tcc cat gtt   1158
122 Ser Lys Thr Glu Ile Glu Met Asp Gly Lys Val Val Asn Ser His Val
123             285                 290                 295
125 gac agt ttc tta gat gga gcc ctg gtt ctg gag gtt gat gag agg gac   1206
126 Asp Ser Phe Leu Asp Gly Ala Leu Val Leu Glu Val Asp Glu Arg Asp
127             300                 305                 310
129 caa gat gat gaa gaa gcc agg cga gaa atg gct agg att ctg aag gaa   1254
130 Gln Asp Asp Glu Glu Ala Arg Arg Glu Met Ala Arg Ile Leu Lys Glu
131     315                 320                 325
133 ctc aag cag aag cat cca gag aag gaa ata gag caa tta ata gaa tta   1302
134 Leu Lys Gln Lys His Pro Glu Lys Glu Ile Glu Gln Leu Ile Glu Leu
```

RAW SEQUENCE LISTING   DATE: 07/24/2001
PATENT APPLICATION: US/09/901,419   TIME: 10:53:13

Input Set : A:\Umo15311.app
Output Set: N:\CRF3\07242001\I901419.raw

```
135 330                   335              340                   345
137 gcc aat tac caa gtc tta agt cag cag caa aaa agt cga gcg ttt tac   1350
138 Ala Asn Tyr Gln Val Leu Ser Gln Gln Gln Lys Ser Arg Ala Phe Tyr
139             350              355                   360
141 cgt att caa gct acc cgc ctg atg acc gga gca ggc aac att tta aag   1398
142 Arg Ile Gln Ala Thr Arg Leu Met Thr Gly Ala Gly Asn Ile Leu Lys
143             365              370                   375
145 agg cat gca gca gac caa gcc agg aaa gct gtc agc atg cat gag gtc   1446
146 Arg His Ala Ala Asp Gln Ala Arg Lys Ala Val Ser Met His Glu Val
147         380                  385                  390
149 aac acg gaa gtg gct gaa aat gac cct gtc agt aag atc ttc ttt gaa   1494
150 Asn Thr Glu Val Ala Glu Asn Asp Pro Val Ser Lys Ile Phe Phe Glu
151     395                  400                  405
153 caa ggg aca tat cag tgt ctg gag aac tgt ggc aca gta gcc ctg acc   1542
154 Gln Gly Thr Tyr Gln Cys Leu Glu Asn Cys Gly Thr Val Ala Leu Thr
155 410                  415                  420                  425
157 att atc cgc aga ggt ggt gat ttg acc aac act gtg ttt gtt gac ttc   1590
158 Ile Ile Arg Arg Gly Gly Asp Leu Thr Asn Thr Val Phe Val Asp Phe
159             430                  435                  440
161 aga aca gag gat ggc aca gcc aat gct gga tct gat tac gaa ttt acc   1638
162 Arg Thr Glu Asp Gly Thr Ala Asn Ala Gly Ser Asp Tyr Glu Phe Thr
163             445              450                  455
165 gaa gga act gtg gtc ttt aag cct ggt gag acc cag aag gaa atc aga   1686
166 Glu Gly Thr Val Val Phe Lys Pro Gly Glu Thr Gln Lys Glu Ile Arg
167         460                  465                  470
169 gtt ggc atc att gat gat gac atc ttt gag gag gat gag aat ttc ctt   1734
170 Val Gly Ile Ile Asp Asp Asp Ile Phe Glu Glu Asp Glu Asn Phe Leu
171     475                  480                  485
173 gtg cat ctc agc aac gtc aaa gta tct ttg gaa gcc tcg gaa gac ggc   1782
174 Val His Leu Ser Asn Val Lys Val Ser Leu Glu Ala Ser Glu Asp Gly
175 490                  495                  500                  505
177 atc ctg gaa gcc agt cat gtc tct acc ctt gct tgc ctg gga tcc ccc   1830
178 Ile Leu Glu Ala Ser His Val Ser Thr Leu Ala Cys Leu Gly Ser Pro
179             510                  515                  520
181 tcc act gcc acc gtg act att ttt gat gat gac cat gct ggc atc ttt   1878
182 Ser Thr Ala Thr Val Thr Ile Phe Asp Asp Asp His Ala Gly Ile Phe
183             525                  530                  535
185 act ttt gag gaa ccg gtg act cat gtg agt gag agc att ggc atc atg   1926
186 Thr Phe Glu Glu Pro Val Thr His Val Ser Glu Ser Ile Gly Ile Met
187         540                  545                  550
189 gag gtg aaa gtt ctg aga aca tct gga gca cgt gga aat gtt atc gtt   1974
190 Glu Val Lys Val Leu Arg Thr Ser Gly Ala Arg Gly Asn Val Ile Val
191     555                  560                  565
193 ccc tat aag acc att gag ggg acc gcc aga ggt gga ggg gag gac ttt   2022
194 Pro Tyr Lys Thr Ile Glu Gly Thr Ala Arg Gly Gly Gly Glu Asp Phe
195 570                  575                  580                  585
197 gag gac aca tgc gga gag ctc gag ttc cag aat gac gaa att gtc aaa   2070
198 Glu Asp Thr Cys Gly Glu Leu Glu Phe Gln Asn Asp Glu Ile Val Lys
199             590                  595                  600
```

RAW SEQUENCE LISTING   DATE: 07/24/2001
PATENT APPLICATION: US/09/901,419   TIME: 10:53:13

Input Set : A:\Umo15311.app
Output Set: N:\CRF3\07242001\I901419.raw

```
201 aca ata tca gtc aag gta att gat gat gag gag tat gag aaa aac aag   2118
202 Thr Ile Ser Val Lys Val Ile Asp Asp Glu Glu Tyr Glu Lys Asn Lys
203         605             610             615
205 acc ttc ttc ctt gag att gga gag ccc cgc ctg gtg gag atg agt gag   2166
206 Thr Phe Phe Leu Glu Ile Gly Glu Pro Arg Leu Val Glu Met Ser Glu
207         620             625             630
209 aag aaa gcc ctg tta ttg aat gag ctt ggt ggc ttc aca ata aca ggg   2214
210 Lys Lys Ala Leu Leu Leu Asn Glu Leu Gly Gly Phe Thr Ile Thr Gly
211         635             640             645
213 aaa tac ctg tat ggc cag cct gtc ttc agg aaa gtt cat gct aga gaa   2262
214 Lys Tyr Leu Tyr Gly Gln Pro Val Phe Arg Lys Val His Ala Arg Glu
215 650             655             660             665
217 cat cca ctc ccc tct act ata atc acc atc gca gat gaa tat gat gac   2310
218 His Pro Leu Pro Ser Thr Ile Ile Thr Ile Ala Asp Glu Tyr Asp Asp
219             670             675             680
221 aag cag cca ctg acc agc aaa gag gag gaa gag agg cgc att gcg gaa   2358
222 Lys Gln Pro Leu Thr Ser Lys Glu Glu Glu Glu Arg Arg Ile Ala Glu
223         685             690             695
225 atg ggg cgc ccc att ctg gga gag cac acc aga ctg gag gtg atc att   2406
226 Met Gly Arg Pro Ile Leu Gly Glu His Thr Arg Leu Glu Val Ile Ile
227         700             705             710
229 gaa gaa tcc tac gag ttc aag agt acc gtg gac aaa ctg att aag aag   2454
230 Glu Glu Ser Tyr Glu Phe Lys Ser Thr Val Asp Lys Leu Ile Lys Lys
231     715             720             725
233 aca aac cta gcc ctc gtg gtt ggg acg aac agc tgg aga gag cag ttc   2502
234 Thr Asn Leu Ala Leu Val Val Gly Thr Asn Ser Trp Arg Glu Gln Phe
235 730             735             740             745
237 atc gag gcg atc act gtc agt gct ggg gaa gat gac gat gac gac gaa   2550
238 Ile Glu Ala Ile Thr Val Ser Ala Gly Glu Asp Asp Asp Asp Asp Glu
239             750             755             760
241 tgt ggg gag gag aag ctg ccc tcc tgt ttt gac tac gtg atg cac ttt   2598
242 Cys Gly Glu Glu Lys Leu Pro Ser Cys Phe Asp Tyr Val Met His Phe
243         765             770             775
245 ctg act gtg ttc tgg aag gtc ctc ttc gcc ttt gtc ccc ccg aca gag   2646
246 Leu Thr Val Phe Trp Lys Val Leu Phe Ala Phe Val Pro Pro Thr Glu
247         780             785             790
249 tac tgg aac ggc tgg gcg tgt ttc atc gtc tcc atc ctc atg atc ggc   2694
250 Tyr Trp Asn Gly Trp Ala Cys Phe Ile Val Ser Ile Leu Met Ile Gly
251     795             800             805
253 cta ctg acg gct ttc att gga gac ctc gct tcc cac ttc gcc tgc acc   2742
254 Leu Leu Thr Ala Phe Ile Gly Asp Leu Ala Ser His Phe Ala Cys Thr
255 810             815             820             825
257 atc gcc ctg aag gat tcc gtg acc gcg gtg gtg ttc gtt gcg ctt gga   2790
258 Ile Ala Leu Lys Asp Ser Val Thr Ala Val Val Phe Val Ala Leu Gly
259             830             835             840
261 acc tca gtg cca gac aca ttt gca agc aaa gtg gcc gcc acc cag gac   2838
262 Thr Ser Val Pro Asp Thr Phe Ala Ser Lys Val Ala Ala Thr Gln Asp
263             845             850             855
265 cag tat gcg gat gca tcc ata ggt aac gtc aca ggc agc aac gcg gtg   2886
```

RAW SEQUENCE LISTING   DATE: 07/24/2001
PATENT APPLICATION: US/09/901,419   TIME: 10:53:13

Input Set : A:\Umol5311.app
Output Set: N:\CRF3\07242001\I901419.raw

```
266 Gln Tyr Ala Asp Ala Ser Ile Gly Asn Val Thr Gly Ser Asn Ala Val
267         860             865             870
269 aac gtc ttc ctg ggc atc ggt gtg gcc tgg tcc atc gcc gcc atc tac   2934
270 Asn Val Phe Leu Gly Ile Gly Val Ala Trp Ser Ile Ala Ala Ile Tyr
271     875             880             885
273 cac gcg gcc aac ggg gaa cag ttc aaa gtg tcc cct ggc acg cta gct   2982
274 His Ala Ala Asn Gly Glu Gln Phe Lys Val Ser Pro Gly Thr Leu Ala
275 890             895             900             905
277 ttt tct gtc act ctc ttc acc att ttt gct ttc atc aat gtg ggg gtg   3030
278 Phe Ser Val Thr Leu Phe Thr Ile Phe Ala Phe Ile Asn Val Gly Val
279             910             915             920
281 ctg ctg tat cgg cgg agg cca gaa att gga ggt gag ctg ggt ggg ccc   3078
282 Leu Leu Tyr Arg Arg Arg Pro Glu Ile Gly Gly Glu Leu Gly Gly Pro
283         925             930             935
285 cgg act gcc aag ctc ctc aca tcc tgc ctc ttt gtg ctc ctg tgg ctc   3126
286 Arg Thr Ala Lys Leu Leu Thr Ser Cys Leu Phe Val Leu Leu Trp Leu
287         940             945             950
289 ttg tac att ttc ttc tcc tcc ctg gag gcc tac tgc cac ata aaa ggc   3174
290 Leu Tyr Ile Phe Phe Ser Ser Leu Glu Ala Tyr Cys His Ile Lys Gly
291     955             960             965
293 ttc taa aggaacaatc agatgtagta aatttatata tatatacata tatatatata   3230
294 Phe
295 970
297 cataaaaatt atgtataatg aacagaggaa actggcattt gtcatgtcca cccacctgct 3290
299 gatggaatcc agcttcaaga gcagactctg tactagggcc ggagagagaa ggcatcacct 3350
301 cccgtttccc aggggcgttc gtcttgttga accaggcatg gaggcagggc catctttacg 3410
303 tcagctcagc ccagaagcgg tgtgttctcc ccgggttcat aaatccttaa gttctttgat 3470
305 ttgttttctg tttttgcttg ttttgggtcg gggtagggag gtggttgatg ttagggtttg 3530
307 gttttggttt tgcaggggga agatcagggt ttgtggtcct cttgtgggag gtgatgtcca 3590
309 atctcaatgg taaaaatgga aatcaggaag atgactctcc ctttgcccaa aaactttaaa 3650
311 aattattttg gagtaagaaa ggaaacgggc atggaagaag aaagaagcat gtcttcacca 3710
313 tattactaaa tttcatgcct tatctctgga gtgggagcag aggtgaagtc ctccctccaa 3770
315 gaagaaacag gggagctgga atggagccaa gaagagtcat ggttctagat acagtctgat 3830
317 gtttaaagat acatcgctgc ctggcaccct tgttcaacag gtacaaaaac aacatgccta 3890
319 gattcccagg aacgcacaaa gtcctttctt atctcttcag cgctggactg tgattagcaa 3950
321 ggccctgatt ctgatgttct acacccgctg attccccagc cctcccatcc caaacccctt 4010
323 ctccggaccc tttacccctc gtacaaacag gaagaataac tccattcaaa aagcacacca 4070
325 tcctttccat tcgcatc                                                4087
328 <210> SEQ ID NO: 2
329 <211> LENGTH: 970
330 <212> TYPE: PRT
331 <213> ORGANISM: Bos taurus
333 <400> SEQUENCE: 2
334 Met Leu Gln Phe Ser Leu Ser Pro Thr Leu Ser Met Gly Phe His Val
335   1           5               10              15
336 Ile Ala Met Val Ala Leu Leu Phe Ser His Val Asp His Ile Ser Ala
337             20              25              30
338 Glu Thr Glu Met Glu Gly Glu Gly Asn Glu Thr Gly Glu Cys Thr Gly
339         35              40              45
```

VERIFICATION SUMMARY   DATE: 07/24/2001
PATENT APPLICATION: **US/09/901,419**   TIME: 10:53:14

Input Set : **A:\Umo15311.app**
Output Set: **N:\CRF3\07242001\I901419.raw**

L:10 M:270 C: Current Application Number differs, Replaced Application Number
L:11 M:271 C: Current Filing Date differs, Replaced Current Filing Date